

# Redefining the head–trunk interface for the neural crest

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## Abstract

The head–trunk interface lies at the occipito-cervical boundary, which corresponds to the somite 5/6 level. Previous studies have demonstrated that neural crest cells also behave differently either side of this boundary and that this may be due to intrinsic differences between cranial and trunk crest. However, it is also possible that some of the observed differences between cranial and trunk crest are assigned by environmental cues. We have therefore scrutinised the behaviour of the neural crest cells generated either side of the occipito-cervical boundary in chick and, interestingly, find that both behave in a truncal fashion by traversing the anterior half of their adjacent somites. Furthermore, although not previously described, we find that transient DRGs form opposite somites 4 and 5. Crest cells produced anterior of the somite 3/4 boundary avoid the somites and behave in a non-truncal fashion; these cells populate the pharyngeal arches, and thus contribute to the developing head. We have further shown, via somite transplantations, that differential behaviour of the posterior versus anterior occipital crest is assigned by the somites. If somites 1 to 3 are replaced by trunk somites, then the anterior occipital crest will behave in a truncal fashion by invading the somites. Correspondingly, if these anterior occipital somites are transplanted in place of trunk somites, they perturb the migration of trunk crest. Thus, for the neural crest, the head–trunk interface does not lie at the occipito-cervical boundary, but rather lies at the somite 3/4 level and is defined by the somites. The fact that this boundary lies at the somite 3/4 level in chick is significant as it reflects the more ancient posterior occipital boundary; in fish, only the first three somites contribute to the occipital bone. © 2004 Elsevier Inc. All rights reserved.

**Keywords:** Head–trunk interface; Somite; Neural crest

## Introduction

The occipital–cervical boundary, which lies between somites 5 and 6, marks the interface between the head and the trunk (Gray, 1989). Posterior of this point, the somites form the segmentally organised vertebrae of the backbone, while anteriorly, the occipital somites fuse together to generate part of the occipital bone, a component of the skull. Correspondingly, within the central nervous system, this boundary defines the point of transition from spinal cord to brain. This axial level was also thought to mark a major difference in the behaviour of the neural crest and, indeed, to mark the boundary between cranial and trunk neural crest. Posteriorly, in the trunk, migratory neural crest cells are segmentally organised by the somites and form the dorsal root and sympathetic ganglia. In the head, neural crest migration is markedly different and dorsal root and symp-

thetic ganglia do not form. Transplantation studies, in chick, suggested that the neural crest cells produced anteriorly of the occipito-cervical boundary, the cranial crest, uniquely possessed the ability to generate skeletal derivatives, and that trunk crest was thought to lack this potential (Le Lievre and Le Douarin, 1975; Nakamura and Ayer-le Lievre, 1982).

Recently, however, it has become apparent that cranial and trunk neural crest cells may be less distinct than previously believed. More specifically, it has been shown that trunk neural crest cells can make bone and cartilage when cultured under appropriate conditions, and will contribute to cranial skeletal elements when transplanted directly into the facial primordia (Abzhanov et al., 2003; McGonnell and Graham, 2002). It is therefore possible that differences in the behaviour of the neural crest either side of the occipito-cervical boundary are due to differential environmental cues.

To examine this possibility, we detailed the behaviour of crest cells either side of the occipito-cervical boundary. Surprisingly, we find that those cells generated just anterior of the occipital–cervical boundary behave in a truncal fashion, moving through the anterior halves of their adjacent

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somites. It is only anterior of the somite 3/4 boundary that crest cells begin to act in a non-truncal manner, avoiding the somites. Interestingly, we find that these anterior occipital crest cells populate the pharyngeal arches, and thus contribute to the developing head. We also find that the differences in migratory behaviour between the anterior and posterior occipital crest is not intrinsic but is dictated by the environment. If the anterior occipital crest cells are exposed to a 'truncal' environment, by somite transplantation, they behave in a truncal fashion. Thus, the different behaviours of the posterior and anterior occipital crest are likely the result of differences between the anterior occipital somites and more posterior somites. We find, however, that somites 1 to 3 develop normally in terms of forming both anterior and posterior halves, as well as generating the expected proportions of sclerotome and dermamyotome compartments. Yet, these anterior-most somites, in contrast to all the others, lack *HoxB3* expression, suggesting that it is the precise axial patterning of somites that underpins the deployment of neural crest cells.

## Materials and methods

### Embryos

Fertile eggs were incubated at 38°C in a humidified atmosphere and staged according to [Hamburger and Hamilton \(1951\)](#).

### In situ hybridisation

Embryos were fixed overnight in MEMFA (100 mM MOPS, 2 mM EGTA, 1 mM MgSO<sub>4</sub>, 4% formaldehyde, pH 7.8). Whole-mount in situ hybridisation using digoxigenin probes for chick *Delta-1*, *EphA4* (sek1 homologue), *HoxB1*, *HoxA2*, *HoxB3*, *HoxB4*, *Isl-1*, *Pax-1*, *Pax-3*, *RXRγ* and *Sox10* was carried out as described by [Henrique et al. \(1995\)](#). The protocol was modified for double in situ using digoxigenin *Pax-1* (or *EphA4*) probe and fluorescein *Pax-3* (or *Delta-1*) probe such that the fluorescein probe was detected using anti-FITC antibody (1:4000; Roche) followed by the NBT/BCIP colour reaction, the embryos refixed and the digoxigenin probe subsequently detected using anti-digoxigenin antibody (1:2000, Roche) and a fast-red colour substrate (Sigma). Embryos for sectioning were embedded in gelatin-albumin (0.45% gelatin, 25% albumin, 20% sucrose), fixed with 2.5% glutaraldehyde and sectioned at 50 μm on a vibratome.

### Immunohistochemistry

Embryos were fixed overnight in 4% paraformaldehyde. Whole-mount immunohistochemistry was carried out as described by [Begbie et al. \(2002\)](#) using HNK-1 (1:100, Developmental Studies Hybridoma Bank) and the

anti-neurofilament medium chain antibody (1:10 000; clone RMO-270, Zymed). The embryos were incubated anti-mouse HRP-conjugated secondary antibody (1:200, Dako) overnight at 4°C. Labelled cells were visualised using DAB substrate (Sigma) and photographed using a Zeiss Axioskop microscope.

### DiI labelling

Embryos were injected with 3 mg/ml DiI (Molecular Probes) in DMF. The dorsal neural tube opposite a single somite was injected in chick embryos at Hamburger-Hamilton (HH) stages 9–11. At least three embryos were used for each axial level labelled. Embryos were resealed and incubated for 24 h. Embryos were fixed overnight in MEMFA, then observed on a Zeiss Axioskop microscope using a rhodamine filter. Images were assembled using Adobe Photoshop 5.5 and converted to a format that is friendly to colour-blind people (<http://jfly.iam.u-tokyo.ac.jp/color>).

### Somite transplants

Transplantation of trunk somites to the occipital region: donor embryos with at least 13 somites were removed from the egg, washed in Ringer's solution and pinned dorsal side up in a glass dish coated with Sylgard. A solution of 1 mg/ml dispase in L-15 media (Gibco) was placed on the embryos to enzymatically loosen adhering tissue. The dispase was diluted by adding L-15 media containing 10% FCS after which stages IV and V trunk somites (i.e., not the three most recently formed somites) were dissected free of adjacent neural tube, ectoderm and notochord using flame-sharpened tungsten needles. Orientation of the graft was maintained by leaving a small piece of lateral mesoderm and/or endoderm attached to the somites. These were grafted into host embryos at HH10 in which two of the anterior-most three somites had been removed unilaterally. In one set of experiments, the NT was labelled with DiI at the level of the graft before the donor somites were implanted. The eggs were resealed and incubated overnight then removed and fixed in MEMFA for analysis. As an alternative to using DiI to track the behaviour of crest cells in the grafted region, double in situ hybridisation was then carried out using a FITC-labelled *RXRγ* probe to mark neural crest cells and a DIG-labelled *HoxB4* probe to mark the grafted trunk somites. The anterior border of *HoxB4* expression in the paraxial mesoderm lies approximately at the level of somite 7/8 ([Burke et al., 1995](#)).

Transplantation of occipital somites to trunk: somites 1 and 2 plus adjacent lateral mesoderm were removed from HH9 donor embryos with the aid of dispase treatment, as above. These were grafted into the trunk of host embryos at HH11 or older, in which stages IV and V trunk somites had been removed. After overnight incubation, the embryos were fixed and analysed using double in situ hybridisation with *RXRγ* and *HoxB4* probes.

## Results

### *The migratory behaviour of the occipital neural crest*

To characterise the migration of the occipital crest, embryos were analysed with a range of neural crest markers, including *Sox10* and HNK-1 (Cheng et al., 2000; Tucker et al., 1984). This analysis clearly establishes that neural crest cells begin to emerge from the occipital neural tube at Hamburger-Hamilton stage (HH)10+, and that once they are in the periphery, these cells follow stereotypical paths (Fig. 1). From HH11, the most anterior group of cells can be

seen to migrate ventrally into the space lying between the otic vesicle and the first permanent somite—somite 2. This space arises as a result of the dissociation of the first somite, which initiates from HH10 (Figs. 1A, C, E, G). These cells continue to migrate ventrally towards the pharyngeal arches, and by HH12/13 this crest stream splits and follows the margins of the pharyngeal pouch endoderm (Fig. 1G, arrows). Although this anterior group of crest cells does encounter somites and some of these crest cells seem to traverse the anterior-most somites, 2 and 3, (Figs. 1A, E, G), this small population migrates along the dorsolateral pathway and passes over these somites; somites 2 and 3 are

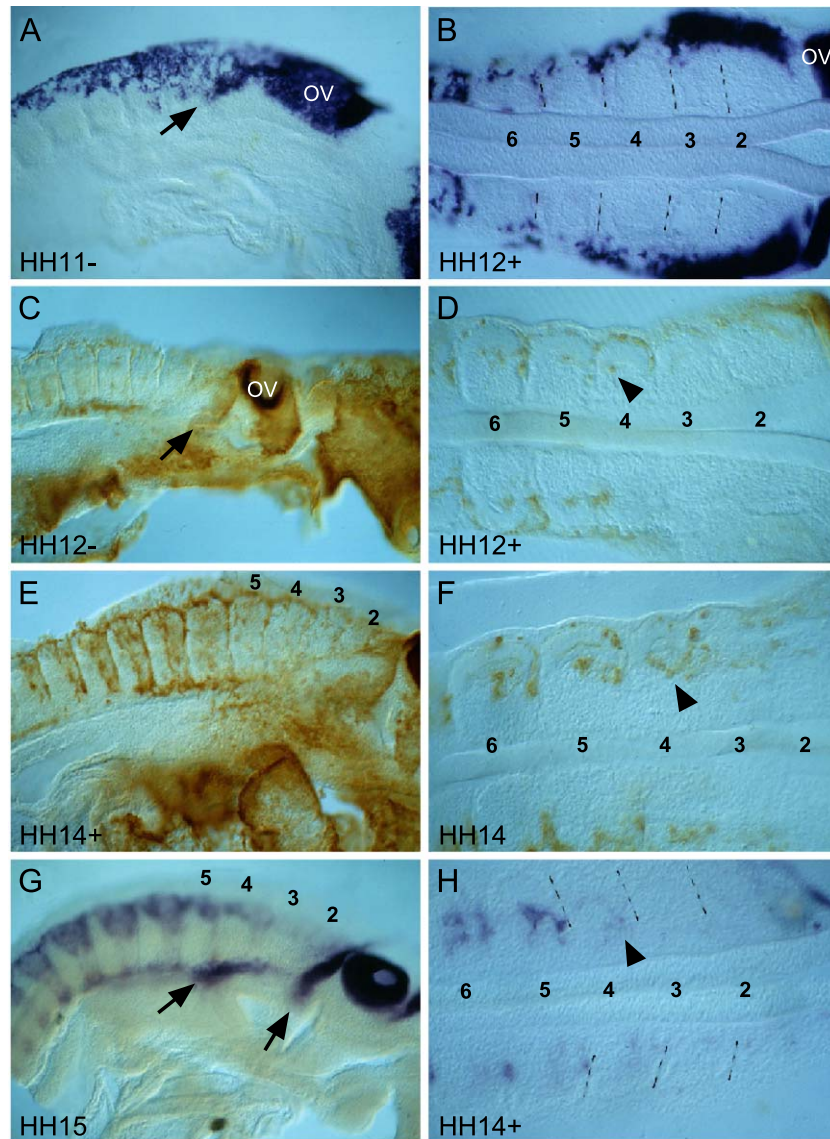


Fig. 1. Different pathways of early neural crest migration within the occipital region. Markers of neural crest cells, *Sox10* expression (A, B, G, H) and HNK-1 antibody binding (C–F), were used to analyse the early migratory pathways of occipital neural crest. Analysis of embryos that were bisected and viewed sagittally (A, C, E, G) shows that anteriorly, there is ventral migration of crest cells into the space lying between the otic vesicle (ov) and the first permanent somite—somite 2, to form the post-otic neural crest stream (arrow). This crest stream migrates towards the posterior pharyngeal arches (PA 3, 4, 6), where it splits and follows the margins of the pharyngeal pouch endoderm. Analysis of embryos that were sectioned longitudinally (B, D, F, H) shows that somites 2 and 3 are devoid of crest. By contrast, crest cells traversing more posterior somites pass through the anterior half sclerotome (arrowheads) and between the somites.



invariably devoid of crest (Figs. 1B, D, F, G). By contrast, in the more posterior occipital region, crest migrates laterally from the neural tube and these cells pass through the anterior half sclerotome of somites 4 and 5, as well as migrating between these somites (Figs. 1B, D, F, H, arrowheads). Thus, these posterior occipital crest cells behave in the same manner as “bona fide” trunk crest cells, such as those that can be seen moving through the anterior half of somite 6, the first cervical somite (Figs. 1B, D, F, H). These results suggest that there is an important transition in neural crest behaviour, which occurs level with the junction of somites 3 and 4.

#### *Fates of the occipital neural crest*

To more closely scrutinise the migratory behaviour of the occipital, and anterior cervical, crest and to analyse their immediate destinations, focal injections of the lipophilic dye, DiI, were used. The dorsal neural tube at specific somite levels was injected at HH9–11, that is, before crest emigration, and the embryos incubated for a further 24 h. The fates of each crest population were then analysed. The most anterior crest, those that were labelled at the level of somite 1, were found to contribute to all three posterior pharyngeal arches (Fig. 2A), as were those arising from opposite somites 2 and 3 (Figs. 2B, C). Thus, the crest stream that fills the posterior arches is composed of a mixture of crest cells arising from the anterior occipital region. Additionally, some crest cells that migrate from the levels of somites 1 to 3 also remain close to the neural tube, and these cells are likely those that will contribute to the superior and jugular ganglia (Figs. 2A–C). Contrastingly, we found that those crest cells that arise opposite somites 4 and 5 do not contribute to the pharyngeal arches, but rather, like those emerging opposite somite 6, migrate through the anterior halves of these somites and move ventrally, and thus behave in a truncal fashion (Figs. 2D–F).

#### *The formation of dorsal root ganglia from the occipital neural crest*

The occipital region is noted for its absence of dorsal root ganglia (DRG) and sympathetic ganglia, and one reason for this could be that crest cells at this axial level fail to populate the anterior sclerotome of the somites. However, as we have shown, this is only partially true; crest cells do populate the anterior sclerotome of somites 4 and 5. We therefore analysed whether these cells attempted to form a DRG. To do this, we employed *Isl-1*, a LIM homeodomain transcription factor that is an early marker of peripheral neurons (Ericson et al., 1992). The characteristically strong *Isl-1* signal that identifies the neuronal cells of the DRGs was observed in the anterior half of somite 8 and similarly in all somites more posteriorly (Fig. 2G; (Avivi and Goldstein, 1999)). Anterior to this are the transient DRG, also known as Froriep's ganglia that occupy a more dorsal position in

avian embryos and which have previously been seen within somites 6 and 7. Thus, all trunk somites have an associated DRG, be they permanent or transient. Interestingly, when we analysed *Isl-1* expression in detail, we found a group of positive cells more anteriorly within somites 4 and 5 in younger embryos (Fig. 2G). In cross-section, it is apparent that the relatively small *Isl-1*-positive ganglia, associated with somites 4 and 5, occur in the typical position of a DRG, lying lateral to the neural tube (Fig. 2H). Thus, the posterior occipital neural crest cells that move through the anterior half of somites do attempt to make a DRG and are thus typically truncal.

#### *The influence of somitic environment on occipital neural crest deployment*

Our results demonstrate that there is a marked transition in the behaviour of the neural crest on either side of the somite 3/4 boundary. Given this fact, it is possible that these different behaviours are the result of the crest perceiving different environmental cues from the somites themselves. To test this, somite transplants were performed. Anterior occipital somites were removed from host embryos, as the neural crest was beginning to migrate, and were replaced with trunk somites from donor embryos (Fig. 3A). The early migration of anterior occipital crest was visualised in several ways. In some of the embryos, the crest cells were highlighted through *RXRγ* expression, and the transplanted somites by virtue of their *HoxB4* expression; occipital somites do not express *HoxB4*. Other embryos were analysed by DiI labelling the neural crest before transplantation. A large number of *RXRγ*-positive, or DiI labelled, neural crest cells were found to migrate into the sclerotome of the transplanted trunk somites when compared to the unoperated control half of the embryo ( $n = 8/11$ ) or moved between the somites ( $n = 3/11$ ) (Figs. 3B–F). Fig. 3D shows a longitudinal section through such a grafted embryo, and it can be seen that the occipital crest cells, highlighted via *RXRγ* expression, are migrating through the anterior half sclerotome. Thus, the anterior occipital crest adopts a truncal mode of migration in the context of trunk somites.

In reciprocal grafts, where the anterior occipital somites were grafted to the trunk (Fig. 3G), the migration of trunk neural crest cells was reordered ( $n = 5/5$ ) (Figs. 3H, I). In these grafted embryos, which were also analysed through *RXRγ* and *HoxB4* expression, the neural crest cells did not enter the somites but either migrated dorsally over the somites, or accumulated between the somites and the neural tube (Fig. 3I). Thus, further supporting the observation that the anterior occipital somites are impenetrable to crest.

#### *Development of the occipital somites*

To determine what differentiates the anterior occipital somites from more posterior somites, a molecular analysis

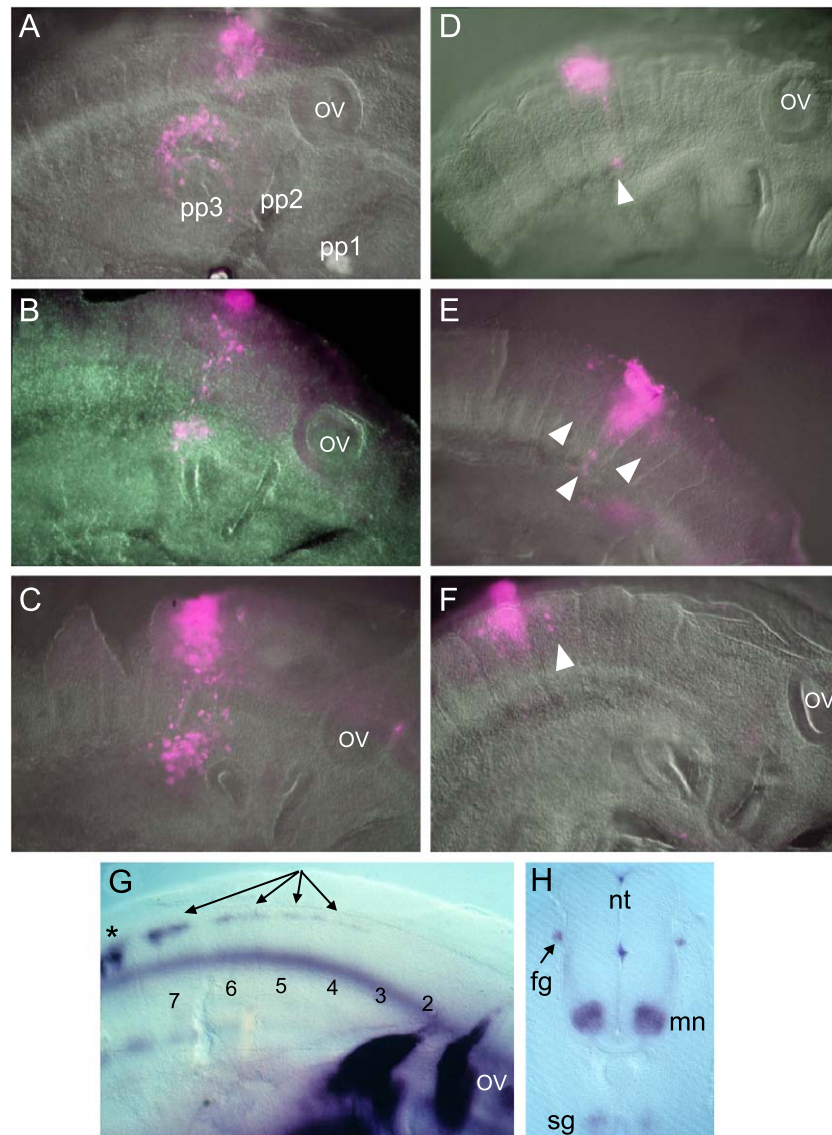


Fig. 2. (A–F) Occipital crest arising opposite somites 1 to 3 utilise different migration routes around the somites compared to crest cells arising at more posterior axial levels. Emerging neural crest that was Dil-labelled at the level of somites 1, 2 or 3 (A–C), move ventrally and were found after 24 h to contribute to all 3 posterior pharyngeal arches. The A–P boundaries of the individual pharyngeal arches (pa2, 3 and 4) are demarcated in lateral view by the appearance of endodermal pharyngeal pouches (pp1–3). Neural crest arising opposite somites 4 and 5 do not contribute to the pharyngeal arches, however, like trunk crest emerging opposite somite 6, are restricted to the anterior halves of the somites (D–F, arrowheads). (G, H) Trunk neural crest derivatives arise in somites 4 and 5. In a HH19 embryo, *Isl-1*-positive cell bodies of the permanent dorsal root ganglia are localised in the anterior sclerotome from somite 8 posteriorly (asterisk) as are those of the transient dorsal root ganglia (Frobiep's ganglia, fg) in somites 6 and 7 (G). In addition, *Isl-1*-positive cells may be detected in occipital somites 4 and 5 (arrows). In cross-section through these posterior occipital somites, the relatively small *Isl-1*-positive ganglia occur in the typical position of a DRG adjacent to the neural tube (nt). Cell bodies of the motor neurons (mn) and sympathetic ganglia (sg) are also *Isl-1*-positive.

was undertaken in which markers were used to answer the following questions: do the first three somites have a sense of anterior–posterior compartmentalisation? Do they have a grossly reorganised sclerotomal and/or dermamyotomal compartments? Is there an upregulation of the inhibitory molecules known to be employed in the posterior half sclerotomes of trunk somites that might prevent crest cells from entering these somites?

*EphA4* and *Delta-1* mark the anterior and posterior compartments of somites, respectively (Bettenhausen et

al., 1995; Nieto et al., 1992), and are expressed transiently in somites as they emerge from the paraxial mesoderm. We find that these genes are expressed appropriately in the first three somites, *EphA4* in the anterior half and *Delta-1* in the posterior, indicating that these somites do have a sense of anterior–posterior compartmentalisation (Figs. 4A–E). *Pax-1* and *Pax-3* are markers of sclerotome and dermomyotome, respectively, (Deutsch et al., 1988; Goulding et al., 1993) and we therefore used these to analyse the organisation of the

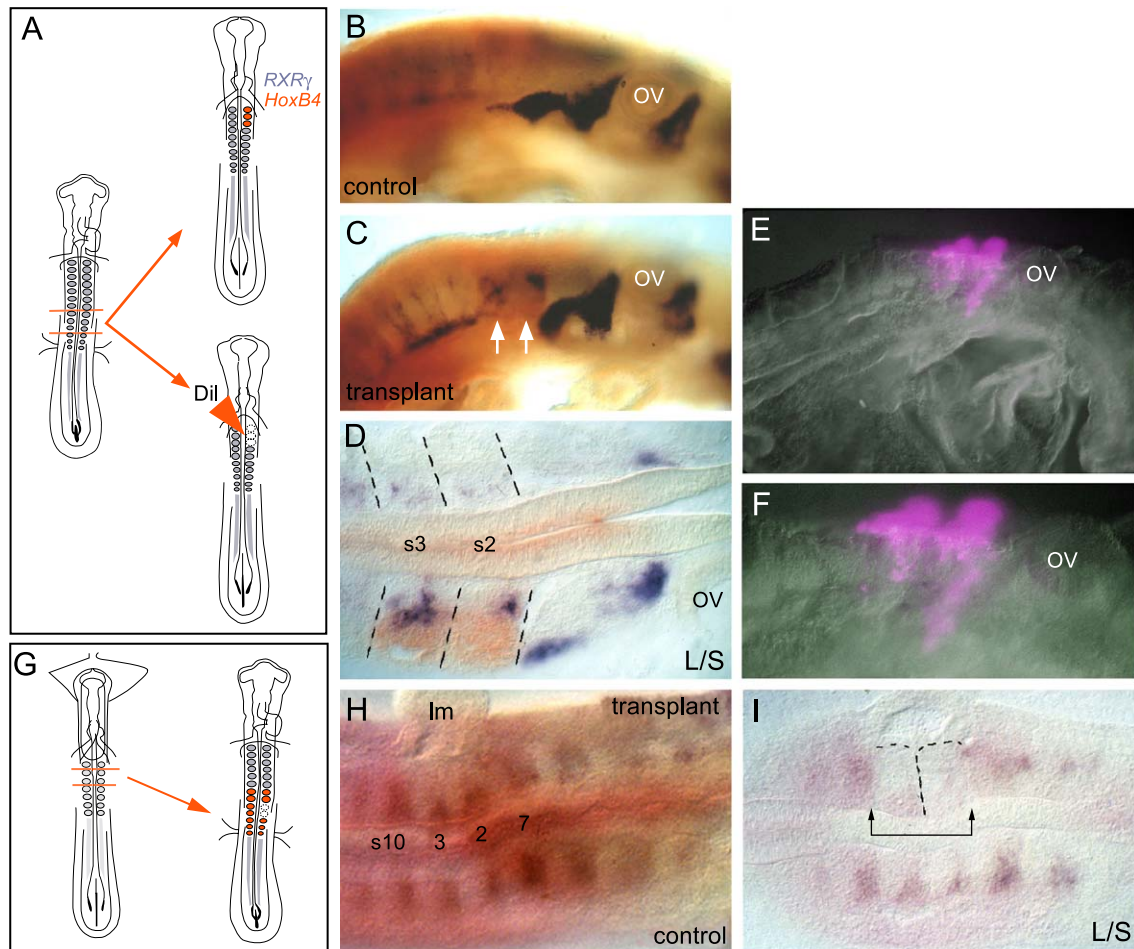


Fig. 3. Somitic environment determines the initial migration path utilised by neural crest. (A, G) schematic representation of the somite transplantation procedures and of the methods used to analyse the resulting migratory behaviour of the neural crest. (B–F) Occipital somites 2 and 3 (*HoxB4*-negative) of a HH11 host embryo were replaced by trunk somites (*HoxB4*-positive) from a HH13 donor embryo. After 24 h, a large number of *RXRγ*-positive neural crest were found to be diverted into the transplanted somites, compared to the unoperated control side of the host embryos (white arrows). (D) In longitudinal section (L/S), the *RXRγ*-positive crest cells were found to move through the anterior half sclerotomes of the transplanted *HoxB4*-positive (red) trunk somites. (E, F) Analysis of *Dil*-labelled occipital neural crest behaviour when exposed to trunk somites: after 24 h, fluorescent cells could be seen in lateral views of the host embryos migrating ectopically through the anterior halves of the transplanted trunk somites. F is a higher power magnification of the image shown in E. (H, I) Analysis of *RXRγ*-positive trunk neural crest behaviour when exposed to (*HoxB4*-negative) occipital somites: after 24 h when whole-mounted embryos were analysed in dorsal view (H) or in longitudinal section (I), crest migration was disrupted. Trunk crest appeared to avoid the occipital somites (marked by arrowed bracket) and instead moved between the neural tube and grafted somites. lm, lateral mesoderm attached to grafted somites that was used for orientation purposes during transplantation.

occipital somites, at stages when crest is known to be traversing them, that is, HH10–HH14 (Figs. 4F–Q). However, we found that these markers were expressed in the anterior-most somites in similar proportions as those found for more posterior somites. Note the presence of *Pax-1*-positive sclerotome and *Pax-3*-positive dermamyotome in somites 1 and 3 at HH10, and their similar distribution when compared with somites 5 and 6 (Figs. 4F–Q). The only noticeable difference is that *Pax-1* expression is downregulated in somite 1 after stage 10, as this somite dissociates (Figs. 4G, H). Finally, we probed the occipital somites for the expression of several molecules that are believed to inhibit crest migration: Sema3A, F-spondin, EphA7, EphrinB1, EphrinB2 and Peanut Lectin binding glycoproteins (Araujo and Nieto,

1997; Davies et al., 1990; Debby-Brafman et al., 1999; Eickholt et al., 1999; Wang and Anderson, 1997). We found that these molecules were not expressed in a manner consistent with them playing an inhibitory role with respect to occipital crest migration (data not shown). From these analyses, the construction and development of the first three somites is similar to trunk somites and does not appear to underlie their impermeability to neural crest cells.

Although the three most anterior somites appear to develop along the same lines as the other somites, their differences could be the result of differential axial specification via Hox gene expression. Members of the first three paralogous groups of Hox genes are known to be expressed in the occipital somites of mice (Kessel and Gruss, 1991),



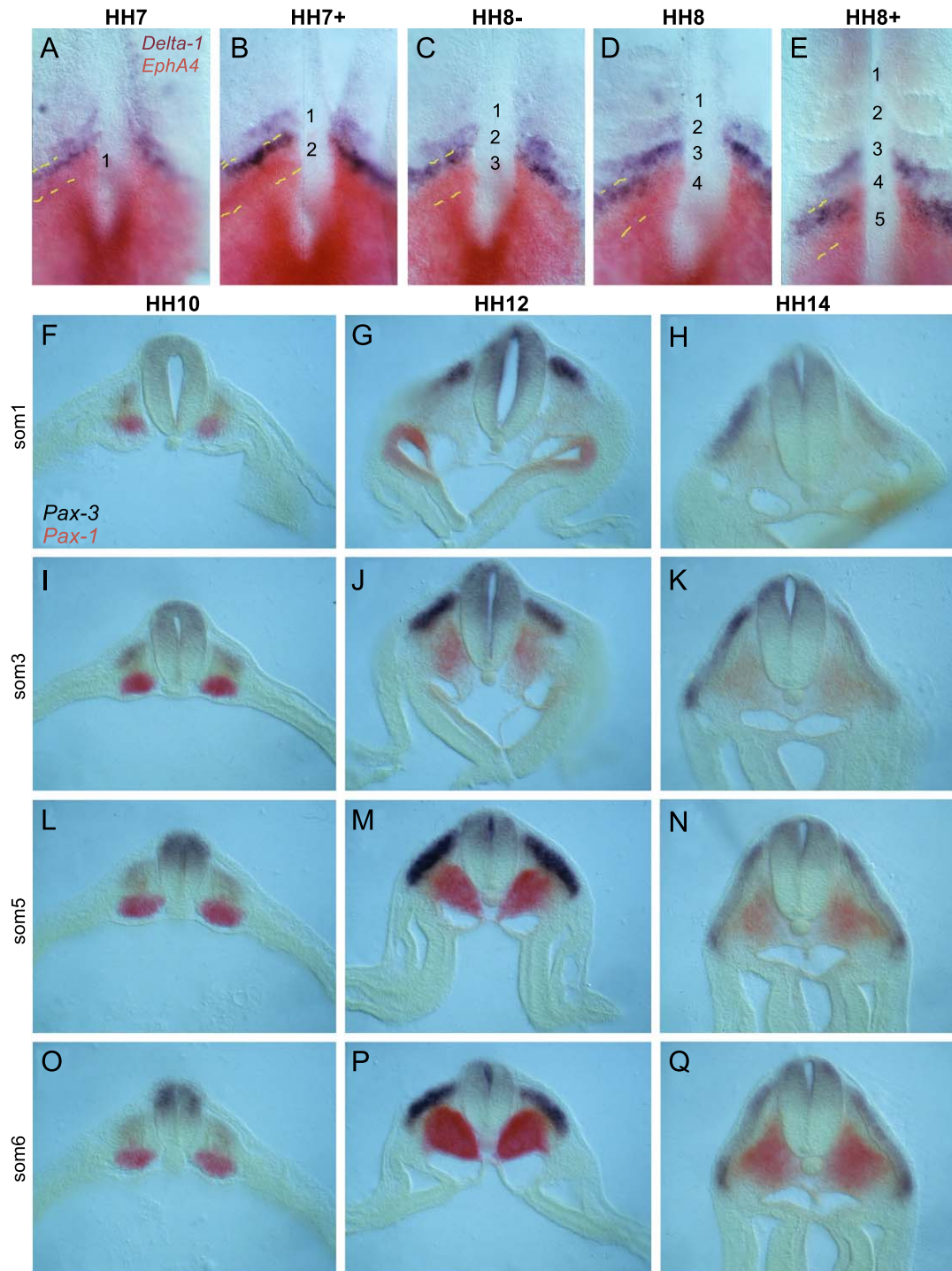


Fig. 4. Segmentation and differentiation of the occipital somites does not differ from more posterior trunk somites. (A–E) Anterior–posterior segmentation of the developing occipital somites was analysed using the transient markers *EphA4* (red) and *Delta-1* (blue). Dorsal view of embryos at stages HH7 to HH9 reveal that *EphA4* is expressed in the anterior domain and *Delta-1* in the posterior domain of the most recently formed somites (indicated by dashed lines). (F–Q) Analysis of the anterior–posterior organisation of the occipital somites at stages when the neural crest is known to be migrating through the occipital region, that is, at HH10 (F, I, L, O), HH12 (G, J, M, P) and HH14 (H, K, N, Q). *Pax-1* (marks sclerotome in red) and *Pax-3* (marks dermomyotome in dark blue) were expressed in the anterior-most somites in similar proportions as those found for more posterior somites. Embryos are viewed in transverse section at the levels of somite 1 (F–H), somite 3 (I–K), somite 5 (L–N) and somite 6 (O–Q).

and we therefore analysed expression of representative genes of each of these paralogous groups in chick embryos. We find that while *HoxB1* and *HoxA2* were expressed in all

somites, *HoxB3* was excluded specifically from the first three somites at all stages analysed and its anterior boundary in the paraxial mesoderm lies within somite 4 (Figs. 5A–F,

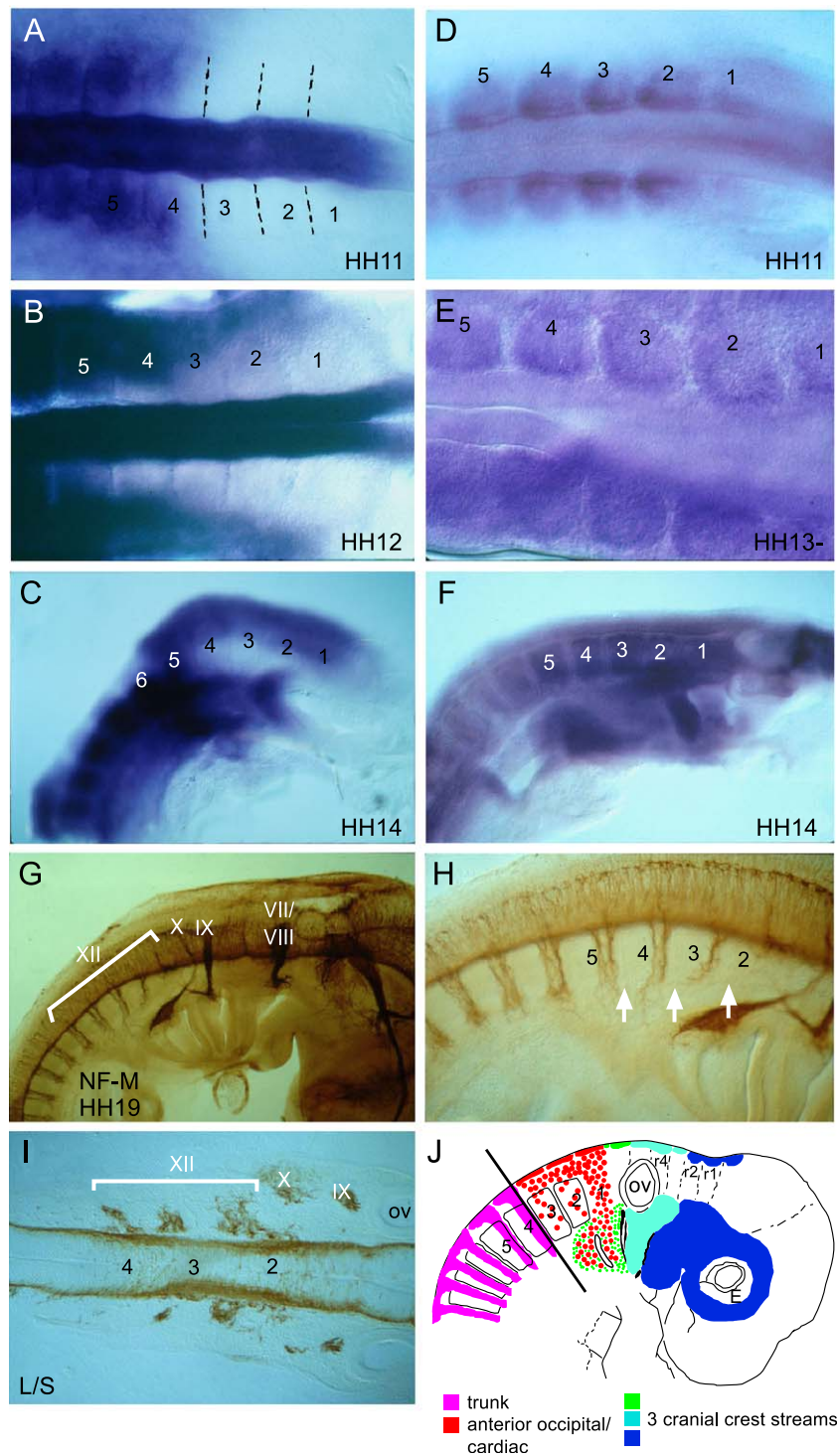


Fig. 5. (A–F) Somites 1 to 3 are differentiated by their lack of *HoxB3* expression. Expression of *HoxB3* (A–C) and *HoxA2* (D–E) in the occipital somites at stages when neural crest cells are known to traverse this region of the embryo, that is, at HH11–HH14. Embryos are viewed dorsally (A, B, D, E) or bisected and viewed laterally (C, F). Positions of the somites are numbered. Note the strong expression of *HoxB3* in the CNS, which extends more anteriorly than in the paraxial mesoderm. (G–I) Rootlets of the hypoglossal nerve are segmentally organised within the anterior halves of the occipital somites. Lateral view of a HH19 embryo that has been stained with an antibody to the neurofilament medium chain (G, H). The cranial nerves VII to XII are indicated. The five pairs of rootlets that contribute to the VII cranial nerve are bracketed in white in G and shown at higher magnification in H, where one can see that each rootlet is positioned in the anterior half of a somite. White arrows indicate the anterior borders of the somites. (I) Longitudinal vibratome section taken through the occipital region showing that the rootlets of the hypoglossal nerve do indeed run through the somites, including the anterior occipital somites. (J) The axial position of the head–trunk interface for neural crest cells lies at the junction of somites 3 and 4. Schematic diagram indicating the migration and immediate destinations of neural crest cells in the anterior half of a chick embryo. A black line is used to indicate the axial level at which neural crest cell behaviour switches to a ‘truncal’ mode. E, eye; r, rhombomere.



and data not shown). *HoxB3* expression, which is relatively weak in somite 4, increases in intensity from somite 5 posteriorly.

In the trunk, both neural crest cells and motor neurons behave in the same manner, only traversing the anterior half sclerotome of each somite. We therefore analysed the projections of the developing hypoglossal nerve to determine if this was also true in the occipital region. The hypoglossal, or 12th cranial nerve, comprises five pairs of rootlets that emerge from ventral exit points in the occipital neural tube and fasciculate ventrally (Fig. 5G). NF-M staining at HH19 reveals that the rootlets are clearly visible and arranged such that each pair is associated with the anterior half of an occipital somite (Fig. 5H, arrows). Longitudinal sections through the occipital region showed that the rootlets move intrasomatically through the somites including somites 2 and 3. Thus, while the neural crest cells that encounter the anterior occipital somites cannot perceive their anteroposterior compartmentalisation, the hypoglossal motor neurons can (Fig. 5I).

## Discussion

Here, we have scrutinised the deployment of the neural crest on either side of the occipital–cervical boundary, which lies at the somite 5/6 level. Interestingly, we find that neural crest cells generated either side of this point behave similarly. The posterior occipital crest, like the cervical crest, migrates through the anterior half of the somites, and thus behaves in a truncal fashion. Indeed, those occipital crest cells that enter somites 4 and 5 attempt to form DRGs. We do find, however, an important transition in the behaviour of the neural crest arising anterior of the somite 3/4 level (Fig. 5J). These crest cells do not enter the somites but migrate anteriorly and then move ventrally between the otic vesicle and the anterior-most somite. These cells also populate the pharyngeal arches, and thus contribute to the developing head. We further demonstrate that the different behaviours of the anterior versus posterior occipital crest are imposed upon these cells by the somites. If trunk somites are grafted in place of somites 1 to 3, then the anterior occipital crest will subsequently behave in a truncal manner, entering these somites and migrating through their anterior halves. Correspondingly, if the anterior occipital somites are transplanted in place of trunk somites, the trunk neural crest migration is perturbed. The impermeability of somites 1 to 3 to the anterior occipital crest may be important to help guide these cells via the post-otic stream towards the pharyngeal arches. We have examined the organisation of somites 1 to 3 to determine how they differ from the other somites, but have found no obvious differences. These most anterior somites have anterior and posterior compartments, generate sclerotome and dermamyotome in expected proportions and do not exhibit unusual expression of previously reported inhibitory molecules. Finally, we find that although neural crest cells do not enter these anterior

somites, motor axons do, and as in the trunk, they traverse the anterior half sclerotome. Therefore, there exists the curious situation in which the motor neurons behave in a truncal fashion, while crest cells from the same axial level do not.

### *The head–trunk interface*

It was long thought that the transition from cranial to truncal behaviour for the neural crest, in amniotes, was coincident with the skeletal interface of the head and trunk, the occipital–cervical boundary. However, we show here that crest cells generated immediately either side of the occipital–cervical boundary both behave in a truncal fashion. Thus, for the neural crest the head–trunk interface does not lie at the occipito-cervical boundary. We did, however, find that there was a major transition in the behaviour of the neural crest either side of the somite 3/4 boundary. In contrast to more posterior crest, those cells born anterior of this point avoid the somites and populate the pharyngeal arches, and as such these cells are cranial. This would suggest that, for the neural crest, the head–trunk interface lies at the somite 3/4 boundary. The lack of alignment between this crest boundary and that of the skeleton would seem somewhat curious, but it is important to note that this “head–trunk” crest boundary is aligned with the skeletal head–trunk boundary of lower vertebrates: in both teleost fish and amphibians, only the first three somites contribute to the occipital bone (Morin-Kensicki et al., 2002). Consequently, the cranial–truncal boundary that we observe for the neural crest in amniotes would appear to reflect the more ancient posterior occipital boundary. Thus, while the evolution of the amniotes involved the recruitment of an additional two somites, to form a more extensive occipital bone, this was not accompanied by a concomitant two somites expansion of head crest domain.

### *The cardiac crest*

The results presented here also have a major impact upon our understanding of the nature of the cardiac crest. It has in the past been suggested that the crest cells emerging opposite somites 1 to 3 were special in that they were the cardiac crest (Kirby et al., 1983), and furthermore that this unique potential was intrinsic (Kirby, 1989). The evidence for this was based on transplantation studies which found that neither trunk nor mesencephalic crest could substitute for cardiac crest in effecting truncal septation (Kirby, 1989). This study suggested that it provided further evidence for intrinsic differences between crest cells from different axial levels. However, as discussed, it is now clear that crest cells from different axial levels do not possess intrinsic differences with respect to the types of derivatives that they can generate. Furthermore, we have also shown that so-called cardiac crest, those cells arising opposite somites 1 to 3, are plastic and will behave in a truncal fashion if presented with trunk somites. Rather, it is more likely that the apparent

intrinsic differences between cardiac crest and other crest populations are due to the manner in which this was assessed. For mesencephalic or trunk crest to behave exactly as cardiac crest, the grafted tissue must fit seamlessly and the transplanted cells exposed to exactly the same environment, both spatially and temporally, for them to be allowed to follow the torturous route from the neural tube, between the somites and otic vesicle, through the pharyngeal arches and finally to the developing heart. Thus, the fact that these transposed crest cells failed to populate the heart is not necessarily a reflection of intrinsic differences between mesencephalic and cardiac crest. However, in keeping with the prevailing views regarding crest development at that time, this was a reasonable conclusion.

#### *Somitic control of head trunk interface for the neural crest*

The fact that it is differences between the occipital somites that act to assign cranial versus truncal behaviour on the crest is consistent with previous studies. It has been shown that if occipital neural tube is transplanted in place of spinal cord, at wing level, then the crest cells produced by the graft are segmentally organised by the trunk somites and form DRGs (Lim et al., 1987). Furthermore, it has been demonstrated that when occipital somites are grafted posteriorly, they do not behave as normal trunk somites and they do not support DRGs (Kant and Goldstein, 1999). In keeping with these previous reports, we also show here that transplanted anterior occipital somites impact upon the migration of trunk crest causing them to take divergent paths. Finally, it is worth noting that the significance of the somites controlling migration of the occipital crest is that deficits in the posterior pharyngeal arches, or in the derivatives of the cardiac crest, could therefore arise as a secondary consequence of alterations to the anterior occipital somites, and not directly as result of modifications to the development of the crest cells themselves.

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